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EFFECT OF DIETARY LEVEL OF PROTEIN, FIBRE AND FAT ON AMINO ACID DIGESTIBILITY IN EARLY-WEANED PIGS

by

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1. INTRODUCTION

The "post-weaning check" period, which is characterized by little or no weight gain and frequently accompanied by diarrhoea, has been hampering the swine industry for years. This period may last from 7 to 14 days depending on nutritional and environmental conditions. To improve the performance of early-weaned pigs, one of the most important considerations is the formulation of diets that supply the required amount of digestible energy and nutrients, including protein and amino acids.

Many studies have been carried out in the area of protein and amino acid digestibility with growing pigs during the last decade. Presently, there is a great deal of interest in amino acid digestibility studies with early-weaned pigs. The digestive system of the piglet, weaned between three and five weeks of age, is still very immature as exemplified by the (relatively) low rate of secretion of gastric HCL and some of the pancreatic enzymes (Corring et al., 1978). The secretion of digestive enzymes will reach its full potential during the first 10 weeks of life (Shields et al., 1980). As a result, the early-weaned pigs will be more limited (compared to pigs of which the digestive system is fully developed) in its capacity to use plant protein supplements.

There is a scarcity of information on the digestibility of protein and amino acids and factors affecting amino acid

digestibility in the early-weaned pigs, in particular when the measurements are based on the ileal analysis method. Recently, however, techniques have become available for the measurement of ileal amino acid digestibilities in the early-weaned pigs (Walker et al., 1986; van Leeuwen et al., 1987, 1988; Freire et al., 1988; Sauer and de Lange, 1992).

In order to obtain more information on factors affecting ileal amino acid digestibilities in early-weaned pigs, a series of studies were carried out. The objectives were to determine the effect of the level of dietary crude protein, fiber and fat on amino acid digestibility. Corn starch-based soybean meal "model" diets were used in these studies.

A. METHODS FOR MEASURING APPARENT ILEAL PROTEIN AND AMINO ACID DIGESTIBILITIES

Amino acid digestibilities can be determined according to the ileal or fecal analysis method. The fecal analysis method measures the amount of each amino acid consumed and excreted in feces. The amino acid digestibilities determined according to the ileal analysis method are calculated based on the intake and amount of each amino acid recovered in digesta collected from the distal ileum.

Ileal Analysis Method

The ileal analysis method should be considered an

improvement over the fecal analysis method. The original studies by Zebrowska (1973) showed that both intact or enzymatically hydrolysed casein infused into the distal ileum of pigs fed a protein-free diet was digested and absorbed; however, the absorbed material was rapidly and almost completely excreted in urine. When casein was given orally, the level of free amino acids in portal blood was high and that of urea low. Other reports (e.g., Wuensche et al., 1982) show that protein or amino acids infused into the large intestine make little or no contribution to the protein status of the animal. According to Sauer and de Lange (1992), some contribution may possibly occur under dietary conditions when nitrogen per se is limiting for the synthesis of the dispensable amino acids, thereby sparing the utilization of the indispensable amino acids.

The series of events that occur when undigested protein, both from dietary and endogenous origin (including peptides and amino acids not absorbed by the end of the small intestine), enters the large intestine have been described by Tanksley and Knabe (1982). A certain proportion of the dietary protein passes through the large intestine and is excreted in feces; the remainder is fermented by the microflora. Nitrogen will be either absorbed, primarily in the form of ammonia (a small proportion in the form of amines and amides), or incorporated into microbial protein. Some of the microbial protein will be digested and the nitrogen absorbed, primarily

in the form of ammonia. The remainder will be excreted in feces. The fate of endogenous protein is likely to be similar to that of dietary protein. Additional evidence of bacterial fermentation in the large intestine is shown by the relatively large amount of bacterial nitrogen that is present in feces. Mason (1984) showed that bacterial nitrogen can account for 62 to 76% of the total nitrogen in feces. The factors that affect the microbial activity in the large intestine, including the availability of fermentable carbohydrates, were previously discussed by Mason (1984) and Sauer and Ozimek (1986).

Convincing evidence that the ileal rather than fecal analysis method should be used for determining protein and amino acid digestibilities was provided by Dierick et al. (1988) in studies in which the performance of pigs was related to the digestibility. There was a higher correlation between average daily gain and ileal rather than fecal protein digestibility (r = .76 vs r = .34). In the same order, for feed conversion ratio (kg feed consumed/kg carcass gain), the correlation coefficients were -.87 and -.65, respectively. These results further support the view that nitrogen absorbed in the large intestine does not contribute significantly to protein synthesis.

Amino acid digestibility coefficients obtained by the fecal analysis method are, for most amino acids in most feedstuffs, higher than those determined by the ileal analysis method. However, net synthesis of methionine and lysine in the

large intestine has been reported in some studies (Zebrowska, 1978; Low, 1980; Sauer et al., 1982; Tanksley and Knabe, 1982). Therefore, depending on the amino acid and on the feedstuff, digestibilities obtained by the fecal analysis method usually overestimate (which is usually the case) or underestimate those obtained by the ileal analysis method. Lysine, the sulphur-containing amino acids, threonine and tryptophan can be considered the most important amino acids in practical diet formulation, as these are often first, second, or third limiting in many feedstuffs. Of these amino acids, cysteine, threonine and tryptophan usually disappear to a large extent in the large intestine.

Methods for Ileal Digesta Collection

Many studies have been carried out in recent years with surgically modified pigs to determine the digestibilities of amino acids at the end of the small intestine. There are several approaches to determine the ileal amino acid digestibilities. The most commonly used method is via collection of digesta from a simple T-cannula, placed 5 to 10 cm anterior to the ileocecal valve. There are a number of concerns with this approach, including the ability to obtain representative samples and the possible shortcomings of the digestibility markers. Furthermore, to obtain representative samples, the frequency and duration of sampling in relation to the time and frequency of feeding should be considered. Other

factors, such as the internal diameter of the cannula, the dry matter content and viscosity of the digesta, the fiber content of the diet, the mesh size of the screen through which the ingredients of the diet were ground, and the amount of digesta to be collected, should also be taken into account. An interesting modification to possibly improve representative sampling of digesta is to use an intestinal suction tube (Dierick et al., 1983). On the other hand, the use of a simple T-cannula (instead of a re-entrant cannula) avoids the transection of the small intestine and the disruption of the myoelectric complex. A more normal physiological state is thus maintained.

Because of the uncertainties associated with obtaining representative samples via a simple T-cannula and the shortcomings of the digestibility markers, studies with pigs fitted with re-entrant cannulas are often preferred, depending on the objectives and experimental conditions of the studies. The pigs can be fitted with either re-entrant cannulas placed in the distal ileum (ileo-ileo re-entrant cannula) or ileo-caecal re-entrant cannulas. Ileo-ileo re-entrant cannulas are usually placed approximately 30 to 40 cm anterior to the sphincter. Ileo-caecal cannulas are placed in the ileum (5 to 10 cm anterior to the sphincter) and caecum. There seems to be less blockage of digesta in pigs fitted with ileo-caecal cannulas, in which digesta bypass the sphincter, than in pigs fitted with ileo-ileo re-entrant cannulas.

Studies with pigs fitted with re-entrant cannulas are often hampered by problems that result from blockage of digesta. The pigs will go off feed abruptly. Although frequent inspection and cleaning of the cannulas reduces the incidence of blockage, this remains a major problem. Blockage occurs more frequently with increasing particle size, fiber content, feed intake, and viscosity of the digesta. Therefore, in many studies in which re-entrant cannulas were used, the diets were finely ground (<1-mm mesh screen) and feed intake restricted (e.g., Braude et al., 1976; Sauer et al., 1977a,b; Taverner et al., 1981).

Problems caused by blockage can be minimized by a constant infusion of a physiological salt solution into the proximal part of the ileo-caecal re-entrant cannula (van Leeuwen et al., 1987). The solution is infused at a rate of 1500 Ml per 24 h for pigs ranging in body weight from 50 to 70 kg. The decrease in dry matter content of digesta passing through the re-entrant cannula likely allows for a smoother transit. This modification makes it possible to determine the ileal digestibilities of amino acids in commercially ground diets. As shown in studies with wheat (Sauer et al., 1977a) and sorghum (Owsley et al., 1981), the particle size of the diet may have a significant effect on ileal amino acid digestibilities. The digestibilities of all the indispensable amino acids were higher ($P<.05$) in finely ground (1 mm mesh screen) than in cracked wheat. Increasing the fineness of

grinding, also increased the amino acid digestibilities in sorghum (Owsley et al., 1981). In addition, with the modification by van Leeuwen et al. (1987), it is easier to maintain a normal dietary intake and to determine the amino acid digestibilities in feedstuffs with a higher fiber content.

Darcy et al. (1980) developed the ileo-colic post-valve (IPV) procedure for total collection of ileal digesta. With the exception of a section surrounding the ileo-caecal sphincter, the entire caecum is removed. The proximal cannula is placed in a pouch constructed from this section; the distal cannula is placed in the colon. This method maintains the integrity of the ileum and preserves the functional role of the ileo-caecal sphincter. Furthermore, this technique allows for sampling of digesta according to their normal time of arrival in the large intestine. The preservation of the functional role of the ileo-caecal sphincter prolongs the retention time of digesta in the small intestine by 60 to 90 minutes, which may benefit the digestion of certain feedstuffs (Darcy et al., 1980). These may include feedstuffs that contain protein of low or medium digestibility and that respond to prolonged enzyme hydrolysis. The apparent ileal nitrogen digestibility of a diet with 8% crude protein was higher in pigs prepared with the IPV procedure (83.7%) than pigs fitted with ileo-caecal re-entrant cannulas (81.7%). The apparent ileal digestibilities of amino acids (in casein and

corn gluten meal, based on comparisons of results in the literature) were higher in pigs prepared with the IAV procedure than pigs fitted with ileo-ileo re-entrant cannulas (Laplace et al., 1985). The lower digestibilities in pigs fitted with ileo-ileo re-entrant cannulae likely result from a reduction in the time available for digestion and absorption, since digesta are collected at a distance of 10 cm or more anterior to the ileo-caecal valve.

Fuller and Livingstone (1982) suggested the ileo-rectal anastomosis technique for rapid routine measurement of amino acid digestibilities. There are several variations on this method, which is often referred to as the ileo-rectal shunt (IRS) procedure. In the first variation, the distal part of the ileum, transected anterior to the ileo-caecal sphincter, is joined by anastomosis to the side of the descending colon, just before the rectum. Although partial atrophy takes place, the large intestine remains functional, as revealed by the excretion of feces-like material every other day. The disadvantages of this variation include contamination of ileal digesta with endogenous material from the large intestine. In addition, ileal digesta may pass into the colon (Picard et al., 1984; Laplace et al., 1985). In the second variation, designed to overcome the disadvantages of the first variation, the large intestine is completely separated from the other parts of the digestive tract such that it is no longer involved in the digestive process. As in the first variation

the large intestine must function, although part of the ileal digesta must be diverted from the large intestine. Diversion of undigested material and digesta can be accomplished by a number of surgical techniques

of the ileum.

Ileal amino acid digestibilities were reported in studies using pigs prepared with the IPV procedure and pigs with ileo-cecal re-entrant cannulae (Stein et al., 1999). Although only the average digestibility coefficients of the amino acids of the terminal ileum (the ileum) protein and four mixtured meals) were reported, the authors stated that there were no differences between the different procedures. On the other hand, Berry-Wilson and Jagtiani (1989) found differences between amino acid digestibilities in pigs prepared with the end IPV procedures depending on the nature of the diet used. There were no differences ($P<.05$) for a standard type diet (69% barley, 15% corn, 15% soybean meal, and 4% alfalfa meal) or wheat bran-enriched diet (15.5% wheat bran, 61.1% corn starch and 9.3% casein); however, there was a trend for higher amino acid digestibilities in the wheat bran diet for pigs prepared with the IPV procedure. The amino acid digestibilities were significantly higher ($P<.05$, with the exception of phenylalanine) in the beet pulp-enriched diet (12.0% beet pulp, 50.7% corn starch and 13.1% casein) for pigs prepared with the IPV procedure. The authors attributed these differences to the preservation of the ileo-cecal sphincter in pigs prepared with the IPV procedure, which may account for an improvement in digestibility as a result of an increase in the retention time of digesta.

Several questions remain to be answered on the

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differences in the apparent dry matter and cellulose digestibilities between intact and cecectomized pigs fed corn-soybean meal diets. Cectomy, however, produced a slight increase in nitrogen digestibility (73.4 vs 75.6%, $P<.05$). Additional advantages of the PVTC procedure include the possibility of measuring ileal digestibilities of commercially ground diets and those which contain ingredients rich in fiber.

Procedures for cannulation of baby pigs are found in the recent literature. Walker et al. (1986) described a technique for inserting a simple T-cannula in pigs at 18 days of age. van Leeuwen et al. (1987) described a technique for placing ileo-caecal re-entrant cannulas in pigs at 21 days of age. Freire et al. (1988) used the IRS method to measure the ileal digestibilities of nutrients in starch-rich diets in pigs from 21 days of age. Studies on the digestion of nutrients in baby pigs are of particular interest, as the digestive system undergoes major physiological and nutritional changes during the first weeks of life until the age of 7 to 10 weeks (e.g., Kidder and Manners, 1978). The use of cannulated early-weaned pigs allows for detailed comprehensive investigations into these changes.

B. EFFECT OF THE DIETARY CONTENT OF CRUDE PROTEIN ON THE DIGESTIBILITIES OF CRUDE PROTEIN AND AMINO ACIDS

Early-weaned pigs face a nutritional stress resulting

from the abrupt shift from milk to plant protein-based diets. Many researchers (e.g., Leibholz, 1981, 1985) reported a better weight gain for early-weaned pigs fed diets containing casein or dried milk than diets in which soybean meal was the primary protein source. Milk protein undergoes rapid proteolysis in young pigs (Braude et al., 1970), whereas soybean meal is not so well digested due to the incomplete development of the digestive enzyme system (Garyl et al., 1989).

One of the factors that determines the apparent protein digestibility of a protein supplement is the protein content of the diet. The apparent protein digestibility increases curvilinearly with the protein content of the diet. As was shown by Eggum (1973) in studies with rats, the increase in apparent protein digestibility is greatest at the lower protein levels with increasing protein content. The increase is negligible at higher levels, which results from the fact that metabolic fecal protein accounts for a smaller proportion of protein in feces with increasing dietary protein content. Similarly, it is expected that apparent fecal as well as ileal amino acid digestibilities will vary depending on the dietary amino acid content. For purpose of comparison of apparent ileal amino acid digestibilities between protein supplements, it is therefore important to determine the level of dietary protein at which the values for ileal amino acid digestibilities exhibit the plateau. As far as pigs are

concerned, there is no information in the literature (even for growing and/or finishing pigs) on this topic.

C. EFFECT OF DIETARY CONTENT OF FIBER ON THE DIGESTIBILITIES OF PROTEIN AND AMINO ACIDS

Dietary fiber is by no means a new term to nutritionists. According to Low (1985), two definitions appear to be useful: (a) "the sum of the polysaccharides and lignin which are not digested by the endogenous secretions of the digestive tract" and (b) "non-starch polysaccharides and lignin". The first represents a broad conceptual definition embracing chemical, physical and physiological aspects of fiber which can not yet be fully measured using existing methods. The second represents an entity that can be measured with existing methods.

Many studies have been carried out with pigs to determine the effect of level and source of fiber on crude protein digestibility. The majority of these studies have shown that the inclusion of fiber in the diet, dependent to a certain extent on the level and source, decreases the digestibility of protein (e.g., Woodman and Evans, 1947; Farrell, 1973; Albers and Henkel, 1979). The decrease in fecal protein digestibility, resulting from the inclusion of fiber, can be ascribed in part to an increase in the amount of bacterial protein voided in feces (e.g., Sauer et al., 1991). The amount

of protein in feces that originates from bacteria is likely dependent on the fermentable energy supply to the microflora in the large intestine (Mason, 1984).

There is a scarcity of information on the effect of fiber on amino acid digestibility, especially when measured with the ileal analysis method, in particular as far as studies with early-weaned pigs are concerned. Just et al. (1980) and Dierick et al. (1983) observed a tendency towards lower ileal digestibilities for crude protein and amino acids with the inclusion of fiber. In certain instances for some of the amino acids there were small but significant ($P<.05$) decreases. Studies by Sauer et al. (1991) also showed a very small effect of fiber on ileal amino acid digestibility in 16% crude protein (from soybean meal) corn starch-based diets in which corn starch was replaced by 10% Alphafloc or barley straw. Zebrowska et al. (1981), on the other hand, found a larger decrease ($P<.05$) in the ileal amino acid digestibilities when 5 or 10% cellulose was included in a barley-starch-fish meal diet. Some of the contradictory findings may be attributed to the "dilution effect" resulting from the inclusion of fiber per se in the diet, which results in a lower dietary protein and amino acid content and, therefore, lower apparent ileal amino acid digestibilities. The results from studies by Just et al.(1980), Dierick et al. (1983) and Sauer et al. (1991), however, do not imply that all sources of fiber have only a minimum effect on ileal nitrogen and amino acid digestibility.

For example, the inclusion of 6% methylcellulose decreased the apparent nitrogen digestibility from 76 to 48% (Murray et al., 1977). The inclusion of 7.5% pectin in a corn starch-based diet formulated to contain 16% crude protein from soybean meal decreased the average ileal digestibilities of the indispensable amino acids from 83.9 to 70.6% (Mosenthin et al., 1987). The results of Murray et al. (1977) suggest that hydrolysis of protein rather than the absorption of the products of digestion is impaired when gel-forming polysaccharides are given.

The decrease in ileal amino acid digestibility, however small, observed in studies by Just et al. (1980), Dierick et al. (1983) and Sauer et al. (1991) might result from the adsorption of amino acids and peptides by fiber, withholding these from absorption, the extent of which depends on the degree of lignification (Mitaru et al., 1984). The inclusion of fiber has also been shown to increase the sloughing of intestinal mucosal cells and to enhance mucus production (Schneeman et al., 1982). In vitro studies by Schneeman (1978) showed adsorption of proteolytic enzymes to fiber and a decrease in the activity of these enzymes. However, in vivo studies with pigs fitted with permanent pancreatic re-entrant cannulas showed no effect of fiber on the secretion (measured in total activities) of trypsin and chymotrypsin (Mosenthin et al., 1987).

D. EFFECT OF DIETARY CONTENT OF FAT ON THE DIGESTIBILITIES OF PROTEIN AND AMINO ACIDS

Fat is often included in diets for pigs because of its physical and nutritional properties. Fat increases the energy density of the diet, facilitates the absorption of fat-soluble vitamins and often improves the palatability of the diet. Furthermore, fat reduces the dustiness of the diet as well as the wear and tear on mixing equipment.

Conflicting reports on the effect of fat on protein digestibility appear in the literatures. Marshall et al. (1959) observed no difference ($P>.05$) in protein digestibility when rats were fed diets containing lactalbumin as the sole source of protein and 3 or 15% fat. Lowrey et al. (1962), in studies with pigs weaned at 3 weeks of age fed corn-based soybean meal and fish meal diets, also observed no difference ($P>.05$) in protein digestibility when 10% fat was added to the diets. Furthermore, no differences were found in protein digestibility in studies with pigs fed corn soybean meal diets that contained 0, 5, 10 or 15% fat (Greeley et al., 1964). On the other hand, Asplund et al. (1960), in studies with pigs weaned at 3 weeks fed corn-based soybean meal, meat scraps and brewers' yeast, observed an improvement ($P<.05$) in protein digestibility when 10% fat was added to the diet. Jorgenson et al. (1985), in studies with growing pigs, also obtained an improvement ($P<.05$) in the apparent protein digestibility (in

addition to lysine, methionine and threonine) when the dietary fat content was increased from 3 to 15%. Furthermore, Tao et al. (1971) observed an improvement ($P<.05$) in fecal digestibilities of many of the indispensable amino acids with broilers fed rapeseed meal diet supplemented with 3.5% fat. It is rather difficult to consolidate the findings by various authors and to explain the underlying physiological and nutritional mechanisms for the different results. However, differences in methodology, age of pigs, diet composition, level and source of fat, and level and source of protein may account for the contradictory findings.

With the exception of studies with growing and finishing pigs by Jorgensen et al. (1985) and Imbeah and Sauer (1991), no studies have been carried out on the effect of fat on ileal amino acid digestibilities. As was reviewed by Sauer and Ozimek (1986), the ileal rather than fecal analysis method should be used for the determination of amino acid digestibilities. The studies by Imbeah and Sauer (1991) showed improvements in the ileal digestibilities of most of the amino acids ($P<.05$) when the dietary level of inclusion of canola oil was increased from 2 to 6 to 10% in a corn starch-based 15% crude protein soybean meal diet. The increases, however, were of a small magnitude. The results reported by Imbeah and Sauer (1991) were in general agreement with studies by Jorgensen et al. (1985) who observed small increases ($P<.05$) in the ileal digestibilities of lysine, methionine and

threonine when the fat level was increased from 3 to 15%. The improvements in ileal digestibilities did not result from a decrease in the rate of passage which was measured at the distal ileum (Imbeah and Sauer, 1991). In theory, a decrease in rate of passage would allow for more time for digestion and/or absorption of amino acids. No studies have been reported in the literature on the effect of fat on amino acid digestibilities in the early-weaned pigs.

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2. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON APPARENT ILEAL AND FECAL DIGESTIBILITIES OF PROTEIN AND AMINO ACIDS IN DIETS FOR EARLY-WEANED PIGS

A. INTRODUCTION

The apparent protein (% N x 6.25) digestibility is influenced by the protein content of the diet. The apparent protein digestibility increases curvilinearly with the protein content of the diet. As was shown by Eggum (1973), in studies with rats, the increase in apparent protein digestibility with increasing protein content is greatest at the lower protein levels; the increase is negligible at higher levels. The latter results from the fact that metabolic fecal protein accounts for a smaller proportion of protein in feces with increasing dietary protein content. Similarly, it is expected that apparent amino acid digestibilities will differ depending on the dietary amino acid content. For the aforementioned reasons, values for apparent digestibility are only valid under strictly standardized conditions, at least with respect to the protein (amino acid) concentration in the diet. Disagreement between apparent protein (amino acid) digestibility values at different laboratories might well be due, in part, to differences in the protein (amino acid) levels in the experimental diets.

Furthermore, as was reviewed several times during the last decade (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek,

1986), the ileal rather than fecal analysis method should be used to determine the digestibilities of amino acids in protein containing ingredients.

The objective of this study was to determine the effect of different levels of dietary crude protein on the ileal and fecal digestibilities of crude protein and amino acids in early-weaned pigs. In recent years, several techniques have become available for the determination of ileal amino acid digestibilities in early-weaned pigs (Sauer and de Lange, 1992). In this study, four corn starch-based soybean meal "model" diets were formulated to contain 16.4, 19.5, 22.5 and 25.5% crude protein, respectively.

B. MATERIALS AND METHODS

Animals

Fourteen Pigs (Yorkshire x Landrace), weaned at 3 weeks of age, were obtained from the University of Alberta swine herd. The pigs were housed individually in metabolic crates (height: 85cm; length: 70cm; width: 65cm) in a barn with automatic temperature control (25 to 28°C) and fed an 18% crude protein starter diet (Sauer et al., 1983) ad libitum. Water was freely available from a low-pressure drinking nipple.

The pigs were fitted with a simple T-cannula at the distal ileum on day 2 and 3 after weaning. Most of the pigs recovered from anaesthesia within 2 to 3 h after surgery.

Following a seven day recuperation period, 12 pigs were selected based on recovery performance and fed the four experimental diets (Table 2-1) according to a balanced two period changeover design (Gill and Magee, 1976). Each experimental period comprised 9 days (5 days adaptation followed by 4 days collection). The pigs were fed the experimental diets at a rate of 5% of the average body weight which was determined 8 h prior to the initiation of each experimental period. The average body weights of the pigs at the beginning of the first and second experimental period were 7.9 ± 1.1 and 9.6 ± 1.2 kg, respectively. The total daily allowance was offered in four meals of equal amounts at 0200, 0800, 1400 and 2000 h, respectively. All the pigs usually consumed their meal allowances.

Preparation of Cannulas

The cannulas were prepared according to procedures adapted from Sauer (1976). A finely polished "T" shape stainless steel mould was heated in a Muffle furnace at 200 to 250°C for 15 minutes. The mould was then immersed in a plastisol solution[1] for 5 to 8 min. Thereafter, the mould covered with coagulated plastisol was heated again. This procedure was repeated 3 or 4 times until the barrel of the cannula reached the proper thickness (approx. 3 mm) and a

[1] Auburn Plastics Engineering, Chicago, IL. 60609.

brown-yellowish colour which ensured proper hardness. The mould with the cannula was then immersed in cold water. As soon as the plastisol enveloping the cannula became firm, an incision was made with a surgical scalpel blade along the flange of the T-cannula. The cannula was immediately removed from the mould thereafter and immersed in cold water for another 30 min.

The retaining ring was prepared by pouring the plastisol solution into an aluminum dish. The dish containing plastisol was heated in the Muffle furnace at 220°C for approximately 5 min. Prior to heating, a glass bottle stopper with the same diameter as the barrel of the cannula was inserted into the centre of the dish to prepare a hole through which the barrel of the cannula could be snugly fitted. The hardened cannula and the retaining ring were trimmed and sanded with an electric sander.

Surgical Procedures and Pre- and Post-operative Care

The animals were fasted for 12 h prior to surgery. Long-acting Liquamycin[2] (0.5 mL) was administered intramuscularly 8 h before surgery. The pigs were brought under general anaesthesia using a gas mixture of halothane[3] and oxygen. The right side of the pig, starting from the second last rib

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[2] Trade mark, contains Oxytetracycline. Rogar/STB Inc., London, Ontario.

[3] Fluothane. Ayerst Laboratories, Saint-Laurent, Quebec.

caudally across the flank region, was shaved with an electric clipper, cleaned with Betadine[4] and draped leaving the surgical area exposed. An incision of approximately 7 cm in length was made parallel and caudal to the last rib leaving a space of approximately 3 cm between the incision and the last rib. The distal ileum was identified by locating the ileo-caecal sphincter. The incision in the ileum was made 5 cm cranial to the ileo-caecal junction. Catgut (2-0)[5] was positioned by aid of a purse-string suture through the serosal layer of the intestine to outline the incision length. A scalpel blade (#25) was used to make an incision between the two parallel sutures. After the flanges of the cannula were inserted into the incision, the suture was tightened. Approximately 2 mm below this suture, another purse-string suture was placed around the base to further secure the cannula. Extruded mucosa was trimmed with a scalpel blade.

A fistula was created between the last two ribs by circumcising a piece of skin and penetrating the muscle layers using finger manipulation and a pair of Rochester Pean Forceps. The barrel of the cannula was then pulled through the fistula. The retaining ring was fixed as close as possible to the skin by tying surgical tape around the barrel of the

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[4] 7.5% Povidone-iodine. Purdue Frederick Inc., Toronto, Ontario.

[5] Chromic absorbable suture. DAVIV + GECK Cyanamid Canada Inc., Baie d'Urfé, Quebec.

cannula. Terramycin[6] (0.5 mL) was administered into the abdominal cavity before the incision was closed,.

The pigs were returned to the same metabolic crates after surgery and fasted for 24 hours. The next day the pigs were provided with 25 g of the starter diet four times daily, at 0200, 0800, 1400 and 2000 h, respectively. The dietary allowance was gradually increased until the pigs consumed the starter diet at a rate of 5% of the average body weight.

During the recuperation period, the crate temperature was maintained at 30 to 32°C by adjusting the infrared heating lamp. The stitches on the skin were removed between 7 and 10 days after surgery. From d 3 onwards the pigs were washed with warm water twice daily around the cannula area. Udderfax[7] was applied under and around the retaining ring to minimize skin irritation.

Experimental Diets

Four corn starch-based diets were formulated to contain four different levels of crude protein (% N x 6.25) with soybean meal, which was solvent-extracted (Table 2-2), as the sole source of protein. Canola oil was included at a level of 3.2% to reduce the dustiness of the diet; dextrose at a level of 10.5% to possibly improve the palatability of the diets.

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[6] Oxytetracycline Hydrochloride. Dominion Vet. Laboratories Ltd., Winnipeg, Manitoba.

[7] Coopers Agropharm Inc., AJAX, Ontario.

Solkafloc was included as source of fiber at a level of 4.3% to facilitate ease of defecation. Chromic oxide was used as the marker for the determination of the digestibilities of the parameters measured. Vitamins and minerals were supplemented to meet and exceed NRC (1988) standards. When necessary, L-lysine, DL-methionine and DL-threonine were supplemented to fulfil their respective NRC (1988) requirements. Soybean meal was ground through a 2-mm mesh screen prior to the preparation of the diets. The formulation of the experimental diets is presented in Table 2-1.

Experimental Design

The general layout of the balanced two-period changeover design is illustrated in Figure 2-1.

For this experiment with 4 treatments (T) represented by A, B, C and D, the basic plan required T x (T-1)/2 = 6 Latin squares of size 2. Each square contained 2 randomly selected animals as rows and 2 periods as columns. Therefore, the complete experiment required T x (T-1) = 12 animals. The design was completely balanced because each treatment was compared with any of the other three treatments in exactly 2 blocks (animals). Periods were orthogonal to treatments. As was pointed out by Gill and Magee (1976) the use of this two-period design is advisable in two situations: (1) Complexity and cost demand that the experiment be as short as possible, yet take advantage of the changeover principle to eliminate

variation among animals. Compared to a Latin square design, this balanced two-period changeover design reduces the duration of the experiment by half which in these particular studies minimizes possible effects resulting from gastro-intestinal development, and (2) Animals from a completely randomized first experiment are to be re-used in a balanced fashion in a second experiment to conserve resources and reduce error. The disadvantages of the balanced two-period changeover design is the poor control over changes across periods and its inability to determine interaction effects.

Data analysis was based on the following linear model:

$Y = \mu + T_i + P_j + B_k + \epsilon_{ijk}$

$(i = 1, 2...T; j = 1, 2; k = 1, 2,...T(T-1))$.

Where T_i = fixed effect of treatment.

P_j = fixed effect of experimental period.

B_k = random effect of an animal.

ϵ_{ijk} = systematic error with N(0,1).

Procedures for Collection of Ileal Digesta and Feces

The collection of feces was initiated at 0800 h on day 6 of each period and continued for 48 h. The collection was extended for another 12 h for pigs that did not provide adequate amounts of feces. Ileal digesta were collected for 24 h: 12 h on d 8 (0800 h to 1400 h and every alternate 6 h thereafter) and 12 h on d 9 (1400 h to 2000 h and every alternate 6 h thereafter). The procedure for the collection of

digesta was adapted from Sauer (1976) with minor modifications.

Soft plastic tubing (20 x 4 cm), sealed at one end and filled with 10 Ml of 4% formic acid to stop bacterial activity, was attached to the barrel of the cannula and tightened with an elastic Velcro tape. Before the collection tube was attached, the inside of the barrel of the cannula was flushed with saline to facilitate the flow of digesta. Feces and digesta were frozen at -28°C immediately following collection. Samples were pooled leaving one sample of feces and digesta for each pig in each period.

Chemical and Statistical Analyses

Feed samples were taken each time when the meal allowances were weighed out and finally pooled for each dietary treatment. Samples of diets, digesta and feces were freeze-dried, ground in a Wiley mill through a 0.5 mm-mesh screen and mixed before subsamples were taken for analysis.

Analyses of dry matter and nitrogen were carried out according to AOAC (1980). Chromic oxide analyses on feed, feces and digesta were determined according to Fenton and Fenton (1979). Amino acid analyses were carried out according to HPLC procedures described by Jones and Gilligan (1983) using a Varian 5000 liquid chromatography. The samples were hydrolysed for 24 h with 6 N HCL prior to HPLC procedures. Analyses for neutral-detergent fiber (NDF) and acid-detergent

fiber (ADF) were carried out according to principles outlined by Goering and Van Soest (1970). Analyses of the diets were carried out in triplicate; analyses of digesta and feces in duplicate. The partial chemical and amino acid content of soybean meal and the experimental diets are presented in Tables 2-2 and 2-3, respectively.

Data were subjected to statistical analysis using the General Linear Model (GLM) of SAS (1986). Means of treatments and experimental periods were compared using the Student-Newman-Keuls multiple range test procedure. Linear, quadratic and cubic effects of the treatment means were analyzed according to the orthogonal polynomial regression procedure (Steel and Torrie, 1980).

C. RESULTS AND DISCUSSION

The apparent ileal and fecal digestibility coefficients of dry matter, crude protein and amino acids of the experimental diets are presented in Tables 2-4 and 2-5, respectively.

There were significant differences ($P<.05$) between the ileal dry matter digestibilities among the treatments. There was a linear decrease as the dietary crude protein content was increased from 16.4 to 25.5%. This decrease was the direct result of replacing a highly digestible nutrient source (corn starch) by one of lower digestibility (soybean meal).

There were no differences (P>.05) in the ileal digestibilities of crude protein and amino acids among the dietary treatments. The average ileal digestibilities of the indispensable amino acids between the treatments ranged from 83.5 ± 3.5% to 82.3 ± 4.2% for diets containing 16.4 and 25.5% crude protein, respectively. On the other hand, as the dietary crude protein level increased from 16.4 to 25.5%, there was a linear decrease (P<.05) in the ileal digestibilities of the majority of the amino acids. This decrease, with an increase in dietary protein content, although of a small magnitude, may also result from a decrease in the efficiency of protein digestion and/or amino acid and peptide absorption. Since the absorption of free amino acids through the intestinal villi is an active process, involving a multiplicity of transport systems (Alpers, 1987), it is possible that the total supply of amino acids and peptides may exceed the capability of absorption. Furthermore, as was shown in Table 2-3, the dietary content of NDF and ADF slightly increased as the content of soybean meal was increased from 34.8 to 54.2% in the diets. The increased fiber content may also contribute to the lower digestibilities of protein and amino acids.

In comparison to the other amino acids, the apparent ileal digestibilities of arginine and lysine were usually relatively high whereas those of threonine and glycine were relatively low. These results are in agreement with studies in growing pigs fed corn starch or cereal-based soybean meal

diets (Holmes et al., 1974; Sauer et al., 1982; Knabe et al., 1989; Sauer et al., 1991). The relatively high apparent ileal digestibilities of arginine and lysine and low digestibility of threonine which were observed in these studies tend to support the hypothesis that enzyme specificity is an important determinant of apparent amino acid absorption in the small intestine. As was discussed by Low (1980), of the indispensable amino acids arginine and lysine would be expected to appear first after enzymatic hydrolysis and threonine last based on the known specificity of the proteases and peptidases involved. The relatively low apparent ileal digestibilities of threonine and glycine may, in part, result from their relatively high concentrations in endogenous secretions. Studies by Holmes et al. (1974) and Sauer et al. (1977) showed a relatively high content of threonine and glycine in endogenous protein from digesta collected from the distal ileum of growing pigs fed a protein-free diet. Glycine, a constituent of one of the bile acids, glycocholic acid, accounts for more than 90% of the total of the amino acids secreted in porcine bile juice (Souffrant, 1991). Furthermore, the small intestinal secretions, which include mucins, supply the largest proportion of nitrogen to the endogenous nitrogen secretions in the small intestine (Auclair, 1986). As was shown by Neutra and Forstner (1987), "native" mucin which represents over 95% of mucin glycoprotein is very rich in threonine in addition to serine and proline. The low apparent

ileal digestibility of threonine may also result, in part, from its relatively low rate of absorption. Buraczewska (1979) studied the ability of different parts of the small intestine to absorb amino acids and peptides, using temporarily isolated loops in growing-finishing pigs. Of the indispensable amino acids, using segments of the middle and distal small intestine, the rates of absorption were highest for arginine, methionine, isoleucine and leucine and lowest for threonine and histidine.

There was no effect (P>.05) of dietary treatment on the apparent fecal digestibilities of dry matter, crude protein and each of the amino acids (Table 2-5). In contrast to their respective ileal digestibilities, there was no linear decrease in the fecal digestibilities of the amino acids as the dietary protein levels were increased from 16.4 to 25.5%. Furthermore, these results show that the ileal analysis method, compared to the fecal analysis method, is more sensitive for detecting differences in amino acid digestibilities. The present studies, as was the case in other studies (e.g., Vandergrift et al., 1983; van Weerden et al., 1985), show the modifying and apparent equalizing effect of the microflora on amino acid digestibilities.

The disappearance of dry matter, crude protein and amino acids in the large intestine, expressed in percentage units, is presented in Table 2-6. Of the indispensable amino acids, threonine disappeared to the largest extent, ranging from 11.1

to 12.9 percentage units; lysine to the smallest extent, from 4.1 to 6.1 percentage units. Of the dispensable amino acids, glycine disappeared to the largest extent, from 14.5 to 15.9 percentage units. These results are in general agreement with other studies with growing pigs (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). The relatively low disappearance of lysine was also reported by the aforementioned authors. Actually net synthesis of lysine in the large intestine has been reported in one study (Just, 1979). Furthermore, these studies show once more that of the indispensable amino acids the apparent digestibility of threonine is overestimated to the largest extent when the fecal analysis method is used.

The net disappearance of the parameters measured in the large intestine, expressed quantitatively as grams per kilogram dry matter intake, is presented in Table 2-7. There was an increase in the net disappearance of dry matter, crude protein and each of the amino acids as the crude protein content of the diets was increased. The increases were significant ($P<.05$) for dry matter and most of the amino acids. For each of the diets, there was a larger disappearance of the dispensable than of the indispensable amino acids. Of the indispensable amino acids the disappearance was usually highest for leucine and threonine; of the dispensable amino acids for aspartic acid, glutamic acid and glycine. Protein secreted via pancreatic juice and small intestinal juice

contains a relatively large proportion of aspartic acid, glutamic acid and leucine (Corring and Jung, 1972; Gorczewska, 1979). The relatively large disappearance of these particular amino acids in the large intestine might indicate preferential fermentation of undigested endogenous protein (or amino acids) by the microflora in the large intestine, thereby allowing the host animal to recover a large proportion of endogenous protein in the form of ammonia. Contribution of ammonia to the protein status of the pig might occur under certain dietary conditions, i.e. under conditions when nitrogen per se is limiting for the synthesis of the dispensable amino acids.

The average of the apparent ileal as well as fecal digestibilities of dry matter, crude protein and amino acids of the experimental diets for each experimental period are presented in Table 2-6. The ileal digestibilities of dry matter and all amino acids were higher ($P<.05$) for period 2 than for period 1. Of the indispensable amino acids, the differences ranged from 3.6 percentage units for arginine to 7.7 for threonine. The fecal digestibilities of the parameters measured were also higher in period 2 than in period 1. The differences in fecal digestibilities between the two periods, however, were not significant. The aforementioned results show once more that the ileal analysis method is more sensitive than the fecal analysis method for detecting differences in amino acid digestibilities. The improvement in ileal amino

acid digestibilities with age (body weight) in early-weaned pigs may result from further development of the digestive enzyme system. With the exception of lactase of which the secretion decreases, the secretion of nearly all other digestive enzymes increase until the age of about 7 to 8 weeks (e.g., Kidders and Manners, 1978). Lloyd et al. (1957) in earlier studies reported higher digestibilities of dry matter, crude protein, fat and energy in 7 than in 3 week old pigs.

In summary, there were no differences ($P>.05$) in the apparent ileal amino acid digestibilities between diets containing 16.4, 19.5, 22.5 and 25.5% crude protein from soybean meal. However, there was a linear decrease ($P<.05$) in the ileal digestibilities of the majority of the amino acids as the dietary protein content increased.

FIGURE 2-1. BALANCED TWO-PERIOD CHANGEOVER DESIGN[a]

Animal (block)	Period	
	1	2
1	A	B
2	B	A
3	A	C
4	C	A
5	A	D
6	D	A
7	B	C
8	C	B
9	B	D
10	D	B
11	C	D
12	D	C

[a] A, B, C and D represent the dietary treatments.

TABLE 2-1. FORMULATION (%) OF DIETS WITH DIFFERENT LEVELS OF CRUDE PROTEIN (Exp.1)

	level of crude protein (%)			
	16.4	19.5	22.5	25.5
Ingredients:				
Soybean meal	34.8	41.4	47.9	54.2
Corn starch[a]	41.1	35.0	28.7	22.9
Canola oil	3.2	3.2	3.2	3.1
Dextrose[b]	10.7	10.6	10.5	10.4
Solkafloc[c]	4.3	4.3	4.3	4.3
TM salt	.4	.4	.4	.4
Vitamin premix[d]	1.0	1.0	1.0	1.0
Mineral premix[e]	1.0	1.0	1.0	1.0
Antibiotics[f]	.1	.1	.1	.1
Dicalcium monophosphate	1.1	.9	.7	.5
Calcium carbonate	1.2	1.3	1.5	1.6
Chromic oxide[g]	.5	.5	.5	.5
L-Lysine(78%)	.2	-	-	-
DL-Threonine (50%)	.1	-	-	-
DL-Methionine (98%)	.3	.3	.2	-
Total	100.0	100.0	100.0	100.0

[a] St. Lawrence Starch Company Ltd. Mississauge, Ontario, Canada.
[b] Cerelose Corn Products. Englewood Cliffs, NJ. 07632.
[c] Brown company. Berlin, NH. 03570.
[d] The vitamin mixture provided the following per kg diet: 3300 IU vitamin A; 330 IU vitamin D; 24 IU vitamin E; 0.75 mg vitamin K; 5.25 mg riboflavin; 22.5 mg niacin; 15 mg pantothenic acid; 0.026 mg vitamin B_{12}; 1.5 mg thiamine; 2.25 mg vitamin B_6; 0.075 mg biotin; 0.45 mg folacin; 750 mg choline.
[e] The mineral mixture provided the following per kg diet: 150 mg Fe; 150 mg Zn; 9.0 mg Cu; 0.21 mg I; 0.3 mg Se.
[f] ASP 250 provides Aureomycin 100 IU, Sulfamezathine 100 IU and Penicillin 50 IU.
[g] Chromic oxide powder. Fisher Scientific. Fair Lawn, NJ. 07410.

TABLE 2-2. CHEMICAL ANALYSIS AND PARTIAL AMINO ACID CONTENT OF SOYBEAN MEAL (%)[a]

Dry matter	89.2
Crude protein, (% N x 6.25)	52.8
Ether extract	2.2
Neutral-detergent fiber	7.7
Acid-detergent fiber	5.0
Cellulose	3.9
Amino acids:	
Indispensable	
Arginine	3.74
Histidine	1.32
Isoleucine	2.37
Leucine	3.74
Lysine	3.79
Phenylalanine	2.61
Threonine	2.17
Valine	2.69
Dispensable	
Alanine	2.19
Aspartic acid	5.46
Glutamic acid	9.20
Glycine	2.12
Serine	2.41
Tyrosine	1.79

[a] Dry matter basis.

TABLE 2-3. CHEMICAL ANALYSIS AND PARTIAL AMINO ACID CONTENT (%)* OF DIETS WITH DIFFERENT LEVELS OF CRUDE PROTEIN (Exp.1)

	Level of crude protein (%)			
	16.4	19.5	22.5	25.5
Dry matter	89.7	89.6	89.3	89.4
Crude protein	18.3	21.8	25.2	28.5
Neutral-detergent fiber	6.9	7.4	7.9	8.4
Acid-detergent fiber	5.8	6.1	6.4	6.7
Ether extract	3.8	3.9	3.7	3.9
Amino acids:				
Indispensable				
Arginine	1.42	1.67	1.95	2.05
Histidine	.41	.57	.74	.89
Isoleucine	.96	1.10	1.29	1.41
Leucine	1.56	1.79	2.02	2.20
Lysine	1.47	1.53	1.83	2.15
Phenylalanine	1.00	1.15	1.30	1.42
Threonine	.96	.98	1.12	1.26
Valine	.99	1.16	1.29	1.39
Dispensable				
Alanine	.93	1.07	1.23	1.33
Aspartic acid	2.24	2.63	2.98	3.22
Glutamic acid	4.15	5.08	5.53	6.07
Glycine	.90	1.09	1.35	1.46
Serine	1.10	1.36	1.62	1.79
Tyrosine	.61	.71	.81	.88

* Dry matter basis.

TABLE 2-4. APPARENT ILEAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF CRUDE PROTEIN (Exp.1)

	Level of crude protein (%)				
	16.4	19.5	22.5	25.5	SE[a]
Dry matter[f]	73.6[b]	72.0[c]	69.2[d]	67.9[e]	.36
Crude protein[f]	81.1	80.9	78.4	78.2	1.13
Amino acids:					
Indispensable					
Arginine[f]	90.0	90.2	89.1	89.1	.28
Histidine	83.2	86.3	84.8	86.1	.77
Isoleucine[f]	84.2	84.9	83.2	82.2	.75
Leucine[f]	83.4	83.5	81.2	80.4	.81
Lysine	85.4	84.7	83.9	85.1	1.13
Phenylalanine[f]	82.3	82.8	81.1	80.1	.66
Threonine	77.6	78.1	76.3	76.5	.95
Valine[f]	82.2	83.0	80.3	79.1	.88
Dispensable					
Alanine[f]	80.6	80.9	77.9	77.2	1.24
Aspartic acid[f]	83.1	83.2	81.5	79.8	.96
Glutamic acid[f]	88.1	87.3	84.9	84.0	1.46
Glycine	75.4	75.9	75.0	75.0	1.29
Serine	83.7	85.4	84.7	84.4	.68
Tyrosine[f]	85.0	85.0	82.4	80.5	1.26

[a] Standard error of the mean.
[b,c,d,e] Values in the same row with different superscript letters differ ($P<.05$).
[f] Linear effect ($P<.05$).

TABLE 2-5. APPARENT FECAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF CRUDE PROTEIN (Exp.1)

	Level of crude protein (%)				
	16.4	19.5	22.5	25.5	SE[a]
Dry matter	89.8	89.6	88.1	87.3	.69
Crude protein	89.3	89.0	88.5	88.4	.74
Amino acids:					
Indispensable					
Arginine	95.5	95.5	95.6	94.9	.37
Histidine	93.0	94.0	94.5	94.7	.52
Isoleucine	90.0	89.8	89.8	89.0	.67
Leucine	90.5	90.4	90.0	89.3	.57
Lysine	90.5	90.4	90.0	89.2	.57
Phenylalanine	91.2	91.3	90.9	90.1	.52
Threonine	90.6	89.2	89.1	88.9	.62
Valine	89.7	89.5	89.1	88.1	.72
Dispensable					
Alanine	87.9	87.9	87.3	86.8	.77
Aspartic acid	92.9	93.0	92.6	92.0	.56
Glutamic acid	95.5	95.8	95.4	95.0	.38
Glycine	89.9	90.5	91.0	90.6	.54
Serine	93.3	93.7	93.9	93.5	.41
Tyrosine	91.4	91.1	90.8	89.8	.73

[a] Standard error of the mean.

TABLE 2-6. DISAPPEARANCE[a] OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN THE LARGE INTESTINE OF PIGS FED DIETS WITH DIFFERENT LEVELS OF CRUDE PROTEIN (Exp.1)

	Level of crude protein (%)			
	16.4	19.5	22.5	25.5
Dry matter	16.2	17.5	18.9	19.4
Crude protein	8.2	8.1	10.2	10.3
Amino acids:				
Indispensable				
Arginine	5.5	5.4	6.4	5.8
Histidine	9.8	7.7	9.7	10.7
Isoleucine	5.8	4.9	6.6	6.8
Leucine	7.1	6.9	8.9	8.9
Lysine	5.1	5.7	6.1	4.1
Phenylalanine	8.9	8.5	9.8	10.0
Threonine	12.9	11.1	12.8	12.5
Valine	7.6	6.5	8.9	9.0
Dispensable				
Alanine	7.3	7.0	9.4	9.7
Aspartic acid	9.8	9.8	11.1	12.2
Glutamic acid	7.4	8.5	10.5	11.0
Glycine	14.5	14.6	15.9	15.7
Serine	9.6	8.4	9.3	9.1
Tyrosine	6.4	6.0	8.5	9.2

[a] Percentage units.

TABLE 2-7. DISAPPEARANCE[a] OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN THE LARGE INTESTINE OF PIGS FED DIETS WITH DIFFERENT LEVELS OF CRUDE PROTEIN (Exp.1)

	Level of crude protein (%)				
	16.4	19.5	22.5	25.5	SE[b]
Dry matter[c]	160.2	175.5	192.9	190.8	8.18
Crude protein	14.9	18.1	29.9	26.7	3.74
Amino acids:					
Indispensable					
Arginine	.79	.86	.88	1.09	.22
Histidine[c]	.42	.44	.73	.73	.07
Isoleucine[d]	.57	.55	.89	.85	.13
Leucine[c]	1.14	1.24	1.87	1.80	.07
Lysine[d]	.72[f]	1.01[ef]	1.65[e]	1.27[ef]	.18
Phenylalanine[c]	.90	.98	1.32	1.34	.11
Threonine[d]	1.39	1.10	1.47	1.59	.15
Valine[d]	.77	.76	1.18	1.12	.15
Subtotal	7.10	6.94	9.99	9.79	
Dispensable					
Alanine[d]	.65	.75	1.19	1.16	.17
Aspartic acid[c]	2.24	2.57	3.37	3.71	.32
Glutamic acid[c]	3.06	4.29	5.88	6.42	.84
Glycine[c]	1.33[f]	1.78[ef]	2.19[e]	2.43[e]	.19
Serine[c]	1.07	1.13	1.53	1.53	.12
Tyrosine[d]	.41	.43	.70	.73	.11
Subtotal	8.76	10.95	14.86	15.98	

[a] Grams per kilogram dry matter intake.
[b] Standard error of the mean.
[c] Linear effect (P<.05).
[d] Cubic effect (P<.05).
[e,f] Values in the same row with different superscript letters differ (P<.05).

TABLE 2-8. EFFECT OF EXPERIMENTAL PERIOD ON THE ILEAL AND FECAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF CRUDE PROTEIN (Exp.1)

	Ileal			Fecal		
	1	2	SE[a]	1	2	SE[a]
Dry matter	69.7[c]	71.5[b]	.26	88.4	89.2	.49
Crude protein	79.1	79.5	.83	88.4	89.3	.52
Amino Acids:						
Indispensable						
Arginine	88.2[c]	91.2[b]	.21	95.2	95.6	.26
Histidine	82.9[c]	87.4[b]	.62	93.8	94.3	.37
Isoleucine	81.5[c]	85.8[b]	.53	89.3	90.1	.47
Leucine	79.9[c]	84.2[b]	.59	89.8	90.4	.40
Lysine	82.9[c]	86.6[b]	.86	91.4	91.7	.56
Phenylalanine	79.2[c]	83.9[b]	.49	90.5	91.3	.37
Threonine	73.3[c]	81.0[b]	.66	89.2	89.8	.43
Valine	78.6[c]	83.7[b]	.65	88.8	89.6	.51
Dispensable						
Alanine	76.7[c]	81.6[b]	.91	87.1	87.9	.54
Aspartic acid	79.1[c]	84.6[b]	.71	92.3	93.0	.40
Glutamic acid	84.1[c]	87.9[b]	.99	95.2	95.7	.27
Glycine	71.4[c]	78.8[b]	.93	90.2	90.8	.38
Serine	82.9[c]	86.4[b]	.50	93.4	93.9	.29
Tyrosine	81.6[c]	84.8[b]	.93	90.5	91.2	.52

[a] Standard error of the period mean.
[b,c] Values of ileal digestibilities in the same row with different superscript letters differ (P<.05).

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3. EFFECT OF DIETARY FIBER LEVEL ON APPARENT ILEAL AND FECAL DIGESTIBILITIES OF PROTEIN AND AMINO ACIDS IN EARLY-WEANED PIGS

A. INTRODUCTION

Many studies with growing-finishing pigs have shown a decrease in apparent fecal crude protein and amino acid digestibilities when fiber is included in the diets (Knox et al., 1951; Kass et al., 1980; Partridge et al., 1981; Fernandez and Jorgensen, 1986). There is scarcity of information on the effect of dietary fiber on protein and amino acid digestibilities in early-weaned pigs, in particular when the digestibilities are determined according to the ileal analysis method. As has been reviewed many times during the last decade, the ileal rather than fecal analysis method should be used to determine amino acid digestibility (e.g., Fuller and Reale, 1980; Mason and Palmer, 1980).

In recent years, various techniques have been developed for the determination of ileal amino acid digestibilities in early-weaned pigs. These include the insertion of a simple T-cannula at the ileal level (Stahly et al., 1979), the collection of ileocecal digesta using the tension technique (Fuller et al., 1979), the use of a re-entrant cannula (Reale et al., 1985), and the use of slaughtered animals (Jansman et al., 1989). Although the re-entrant cannulation technique is considered to be the most accurate, it is also the most difficult to

in diets for early-weaned pigs.

The objective of this study was to determine the effect of the level of dietary fiber on the apparent ileal and fecal digestibilities of crude protein and amino acids in diets for early-weaned pigs. Solkafloc was selected as source of fiber and included at four levels (at the expense of corn starch) in a 22.5% crude protein corn starch-based soybean meal "model" diet.

B. MATERIALS AND METHODS

Sixteen pigs (Yorkshire x Landrace), weaned at 3 weeks of age, were fitted with a simple T-cannula at the distal ileum on day 2 and 3 after weaning. The housing conditions, surgical procedures, pre- and post-operative care, cannula design, feeding regimen, experimental design and other experimental conditions were similar to those described in chapter 2.

Following a seven day recuperation period, 12 pigs were selected and fed the four experimental diets according to the cross experimental design outlined in chapter 2. The average body weights of the pigs were 8.8 ± 1.2 kg and 10.7 ± 1.7 kg at the beginning of the first and second experimental periods, respectively.

The formulation of the experimental diets is presented in table 3.1. Solkafloc was included at levels of 0.0, 7.5, 15.0 and 22.5% (at the expense of corn starch) in a 22.5% crude protein corn starch-based diet. The dietary protein was

supplied by soybean meal, which was solvent-extracted. Canola oil, 3.2% , was included to reduce the dustiness of the diets. Dextrose, at a level of 10.5%, was included to possibly improve the palatability of these semi-purified diets. DL-methionine, vitamins and minerals were supplemented to meet or exceed NRC (1988) standards. Chromic oxide was used as marker to determine the digestibilities of the parameters that were measured.

Chemical and statistical analyses were carried out according to the procedures described in chapter 2. The partial chemical and amino acid composition of the experimental diets are presented in Table 3-2.

C. RESULTS AND DISCUSSION

The pigs seemingly remained healthy and consumed their daily allowances throughout the study.

The apparent ileal and fecal digestibility coefficients of dry matter, crude protein and amino acids are presented in Tables 3-3 and 3-4, respectively. The ileal dry matter digestibility decreased linearly ($P<.001$) with increasing dietary fiber content. This observation is in agreement with studies with growing pigs (Fernandez and Jorgensen, 1986; Mosse et al., 1988). This result was quite predictable since cellulose was included in the diet at the expense of corn starch. The decrease in dry matter digestibility therefore is a direct result of replacing a highly digestible carbohydrate

source (in this case corn starch) with one of low digestibility (Solkafloc).

There were no differences (P>.05) in the apparent ileal digestibilities of crude protein when the dietary inclusion of Solkafloc was increased from 4.3 to 10.3%. However, there was a decrease (P<.05) when the level of Solkafloc was increased from 10.3 to 13.3%, corresponding to ADF levels of 12.0 and 14.8%, respectively. The ileal digestibility coefficients of the majority of the amino acids among the dietary treatments followed the same pattern as crude protein digestibility. There were usually no differences (P>.05) in the amino acid digestibilities between diets that contained 4.3, 7.3 or 10.3% Solkafloc. Although there were detectable differences (P<.05) in some instances, they were of a small magnitude. These results are in general agreement with growing pigs reported by Just et al. (1980), Dierick et al. (1983) and Sauer et al. (1991). For example, with the exception of two of the amino acids, Sauer et al (1991) reported no differences (P>.05) in the apparent ileal amino acid digestibilities in diets in which corn starch was replaced by 10% Alphafloc or ground barley straw.

The ileal digestibilities of the majority of the amino acids decreased (P<.05) when the inclusion of Solkafloc was increased from 10.3 to 13.3%. With the exception of histidine, lysine, glutamic acid and glycine, there was a quadratic relationship between the ileal amino acid digestibilities and

the level of inclusion of Solkafloc with a plateau appearing at the 10.3% inclusion level which is equivalent to 12.0% ADF. From these studies it seems that early-weaned pigs are able to consume up to 10.3% Solkafloc in the diet without a depression in ileal amino acid digestibilities. However, there is depression in ileal amino acid digestibilities when Solkafloc inclusion was increased to 13.3%. The decrease in amino acid digestibility, observed at the higher level of Solkafloc inclusion, might result from the adsorption of amino acids and peptides by fiber, withholding these from absorption, the extent of which depends on the degree of lignification (Mitaru et al., 1984). The inclusion of fiber has also been shown to increase the sloughing of intestinal mucosal cells and to enhance mucus production (Schneeman et al., 1982). The aforementioned factors that may result in a measurable increase in amino acid losses during digestion may only take on quantitative importance following the inclusion of a particular level of fiber in the diet; the exact level also being dependent on the source of fiber. In addition, a high fiber content may stimulate contraction of the digestive tract and thus accelerate rate of passage which might result in lower digestibilities.

Compared to the other amino acids, the apparent ileal digestibilities of arginine and lysine were usually relatively high whereas those of threonine and glycine were relatively low. These results are in general agreement with studies with

growing pigs fed corn starch or cereal-based soybean meal diets (Holmes et al., 1974; Sauer et al., 1982; Knabe et al., 1989; Sauer et al., 1991). Possible reasons for the relatively low and high apparent ileal digestibilities of certain of the amino acids were discussed in chapter 2.

As was the case with the ileal digestibility of dry matter, the fecal digestibility also showed a linear decrease ($P<.001$) as the level of dietary fiber was increased. The level of dietary fiber did not affect ($P>.05$) the fecal crude protein and amino acid digestibilities. The results of these studies are in contrast with studies with growing pigs by Mosenthin et al. (1986) and Sauer et al. (1991). Sauer et al. (1991) reported a decrease ($P<.05$) in the fecal digestibilities of crude protein and amino acids when fiber from Alphafloc or ground barley straw was included in a corn starch-based soybean meal diet. This decrease, in part, was due to an increase ($P<.05$) in the quantity of bacterial protein voided in feces. The absence of a decrease in fecal digestibilities with increasing dietary inclusion of Solkafloc is likely due to its low fermentability. Solkafloc, a wood-derived cellulose, represents a slow-fermenting substrate (van Soest and Robertson, 1976; McBurney and van Soest, 1991).

The disappearance of dry matter, crude protein and amino acids in the large intestine, expressed in percentage units, is presented in Table 3-5. Of the indispensable amino acids, threonine disappeared to the largest extent, ranged from 13.5

to 16.5 units; lysine to the smallest extent, from 4.0 to 7.3 units. Of the dispensable amino acids, glycine disappeared to the largest extent. These results are in general agreement with other studies with growing pigs (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986) and with those reported with early-weaned pigs in chapter 2.

The disappearance of crude protein and amino acids in the large intestine, expressed on a quantitative basis as grams per kilogram dry matter intake, is presented in Table 3-6. For the parameters measured, the disappearance was usually largest when the diet containing 13.3% Solkafloc was fed. As was previously mentioned, the inclusion of 13.3% Solkafloc (compared to the lower levels) resulted in a decrease ($P<.05$) in ileal digestibilities. On the other hand, the fecal digestibilities were not affected ($P>.05$). The quantitative disappearance of the individual amino acids followed the same general pattern reported in chapter 2.

The average of the apparent ileal as well as fecal digestibilities of dry matter, crude protein and amino acids for each experimental period are presented in Table 3-7. As opposed to the results reported in chapter 2, the apparent ileal digestibilities of most of the amino acids were lower in period 2 than in period 1. Although the differences were small, these were significant ($P<.05$) for 4 of the indispensable amino acids. With the exception of leucine and lysine, there were no differences ($P>.05$) between the fecal

amino acid digestibilities in period 1 and 2. No explanation can be provided for the difference in results reported in chapter 2 and in this study.

In summary, the inclusion of Solkafloc in diets for early-weaned pigs up to a level of 10.3% had no effect (P>.05) on the ileal amino acid digestibilities in soybean meal. Exceeding this level of inclusion resulted in lower digestibilities (P<.05). The inclusion of Solkafloc had no effect (P>.05) on the fecal amino acid digestibilities in soybean meal.

TABLE 3-1. FORMULATION (%) OF DIETS WITH DIFFERENT LEVELS OF SOLKAFLOC (Exp.2)

	Level of Solkafloc (%)			
	4.3	7.3	10.3	13.3
Ingredients:				
Soybean meal	47.9	47.9	47.9	47.9
Corn starch[a]	28.8	25.8	22.8	19.8
Canola oil	3.2	3.2	3.2	3.2
Dextrose[b]	10.5	10.5	10.5	10.5
Solkafloc[c]	4.3	7.3	10.3	13.3
TM salt	.4	.4	.4	.4
Vitamin premix[d]	1.0	1.0	1.0	1.0
Mineral premix[e]	1.0	1.0	1.0	1.0
Antibiotics[f]	.1	.1	.1	.1
Dicalcium monophosphate	.6	.6	.6	.6
Calcium carbonate	1.5	1.5	1.5	1.5
Chromic oxide[g]	.5	.5	.5	.5
DL-Methionine (98%)	.2	.2	.2	.2
Total	100.0	100.0	100.0	100.0

[a] St. Lawrence Starch Company Ltd. Mississauge, Ontario, Canada.
[b] Cerelose Corn Products. Englewood Cliffs, NJ, 07632.
[c] Brown company, Berlin, NH, 03570.
[d] The vitamin mixture provided the following per kg diet: 3300 IU vitamin A; 330 IU vitamin D; 24 IU vitamin E; 0.75 mg vitamin K; 5.25 mg riboflavin; 22.5 mg niacin; 15 mg pantothenic acid; 0.026 mg vitamin B_{12}; 1.5 mg thiamine; 2.25 mg vitamin B_6; 0.075 mg biotin; 0.45 mg folacin; 750 mg choline.
[e] The mineral mixture provided the following per kg diet: 150 mg Fe; 150 mg Zn; 9 mg Cu; 0.21 mg I; 0.3 mg Se.
[f] ASP 250, provides Aureomycin 100 IU, Sulfamezathine 100 IU and Penicillin 50 IU.
[g] Chromic oxide powder. Fisher Scientific. Fair Lawn, NJ, 07410.

TABLE 3-2. CHEMICAL ANALYSIS AND PARTIAL AMINO ACID CONTENT (%)[a] OF DIETS WITH DIFFERENT LEVELS OF SOLKAFLOC (Exp.2)

	Level of Solkafloc (%)			
	4.3	7.3	10.3	13.3
Dry matter	88.9	89.3	89.8	89.8
Crude protein	25.1	25.4	25.2	25.3
Neutral-detergent fiber	7.9	10.9	13.9	16.8
Acid-detergent fiber	6.4	9.2	12.0	14.8
Ether extract	4.0	3.9	3.9	4.0
Amino acids:				
Indispensable				
Arginine	1.54	1.50	1.49	1.47
Histidine	.53	.53	.56	.53
Isoleucine	1.18	1.19	1.16	1.11
Leucine	1.72	1.77	1.78	1.69
Lysine	2.22	2.16	2.27	2.18
Phenylalanine	1.21	1.22	1.22	1.21
Threonine	.98	.99	1.00	.99
Valine	.98	1.00	.98	.97
Dispensable				
Alanine	1.09	1.09	1.08	1.08
Aspartic acid	2.53	2.56	2.56	2.45
Glutamic acid	4.39	4.46	4.44	4.31
Glycine	1.01	.97	1.01	.98
Serine	1.09	1.11	1.11	1.09
Tyrosine	.78	.77	.77	.76

[a] Dry matter basis.

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TABLE 3-3. APPARENT ILEAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF SOLKAFLOC (Exp.2)

	Level of Solkafloc (%)				
	4.3	7.3	10.3	13.3	SE[a]
Dry matter[b]	67.8[d]	65.4[e]	64.0[e]	59.0[f]	.56
Crude protein	75.9[d]	74.5[d]	74.9[d]	71.0[e]	.53
Amino acids:					
Indispensable					
Arginine[c]	85.9[de]	85.4[e]	86.4[d]	83.1[f]	.23
Histidine	83.7	80.1	82.3	79.3	1.55
Isoleucine[c]	79.3[d]	79.6[d]	80.0[d]	76.1[e]	.44
Leucine[c]	76.1[d]	76.2[d]	77.8[d]	73.2[e]	.42
Lysine	87.4[d]	85.6[e]	88.1[d]	84.6[e]	.46
Phenylalanine[c]	78.9[e]	78.2[e]	80.1[d]	75.6[f]	.33
Threonine[c]	71.4	72.1	72.6	68.4	1.12
Valine[c]	71.3[d]	72.2[d]	72.8[d]	67.7[e]	.49
Dispensable					
Alanine[c]	73.6[d]	72.5[d]	74.1[d]	68.7[e]	.57
Aspartic acid[c]	76.5[d]	75.7[d]	77.7[d]	72.9[e]	.49
Glutamic acid	79.5[d]	78.7[d]	80.5[d]	75.3[e]	.82
Glycine	65.9[d]	60.8[de]	64.4[d]	59.6[e]	1.31
Serine[c]	75.5[de]	74.6[e]	76.8[d]	72.2[f]	.49
Tyrosine[c]	78.7[e]	79.6[e]	82.5[d]	77.7[e]	.81

[a] Standard error of the mean.
[b] Linear effect (P<.001).
[c] Quadratic effect (P<.05).
[d,e,f] Values in the same row with different superscript letters differ (P<.05).

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TABLE 3-4. APPARENT FECAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF SOLKAFLOC (Exp.2)

	Level of Solkafloc (%)				
	4.3	7.3	10.3	13.3	SE[a]
Dry matter[b]	87.6[d]	84.2[e]	80.3[f]	78.8[g]	.45
Crude protein	88.9	86.7	87.1	86.5	.71
Amino acids:					
Indispensable					
Arginine	94.3	92.8	93.7	93.0	.42
Histidine[c]	91.5	90.1	92.3	90.4	.55
Isoleucine	88.5	86.5	87.1	86.2	.67
Leucine	87.8	86.1	87.0	86.0	.62
Lysine	92.4	91.1	92.1	91.9	.45
Phenylalanine	89.9	88.0	89.1	88.3	.56
Threonine	87.2	85.6	86.4	84.9	.81
Valine	85.1	82.7	83.5	82.6	.91
Dispensable					
Alanine	85.9	83.2	84.0	82.5	.82
Aspartic acid	91.3	89.3	90.3	88.2	.82
Glutamic acid	94.1	92.6	93.3	91.9	.55
Glycine[c]	87.8	84.7	86.3	84.6	.75
Serine[c]	90.3	88.9	89.9	88.3	.63
Tyrosine	90.8	89.4	90.8	90.2	.62

[a] Standard error of the mean.
[b] Linear effect ($P<.001$).
[c] Cubic effect ($P<.05$).
[d,e,f,g] Values in the same row with different superscript letters differ ($P<.05$).

TABLE 3-5. DISAPPEARANCE[a] OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN THE LARGE INTESTINE OF PIGS FED DIETS WITH DIFFERENT LEVELS OF SOLKAFLOC (Exp.2)

	Level of Solkafloc (%)			
	4.3	7.3	10.3	13.3
Dry matter	19.8	18.8	16.3	19.8
Crude protein	13.0	12.1	12.1	15.5
Amino acids:				
Indispensable				
Arginine	8.4	7.5	7.2	9.9
Histidine	7.9	10.0	10.0	11.1
Isoleucine	9.2	6.9	7.0	10.0
Leucine	11.7	9.9	9.2	12.9
Lysine	5.0	5.5	4.0	7.3
Phenylalanine	11.0	9.8	9.0	12.7
Threonine	15.8	13.5	13.8	16.5
Valine	13.8	10.6	10.7	14.9
Dispensable				
Alanine	12.4	10.7	9.9	13.8
Aspartic acid	14.7	13.6	12.7	15.3
Glutamic acid	14.5	13.9	12.8	16.6
Glycine	21.9	23.9	21.9	25.1
Serine	14.8	14.3	13.1	16.0
Tyrosine	12.1	9.8	8.4	12.5

[a] Percentage units.

TABLE 3-6. DISAPPEARANCE[a] OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN THE LARGE INTESTINE OF PIGS FED DIETS WITH DIFFERENT LEVELS OF SOLKAFLOC (Exp.2)

	Level of Solkafloc (%)				
	4.3	7.3	10.3	13.3	SE[b]
Dry matter	191.3	171.9	172.8	188.5	10.65
Crude protein	30.9	27.9	31.7	36.1	1.59
Amino acids:					
Indispensable					
Arginine	1.13	1.09	1.12	1.36	.05
Histidine[c]	.43	.59	.56	.53	.04
Isoleucine	.90[de]	.67[e]	.89[de]	1.04[d]	.05
Leucine	1.75	1.59	1.75	2.02	.08
Lysine	1.19	1.16	1.15	1.47	.09
Phenylalanine	1.16[de]	1.03[e]	1.19[de]	1.43[d]	.06
Threonine	1.42	1.21	1.45	1.56	.13
Valine	1.16[de]	.88[e]	1.13[de]	1.35[d]	.06
Subtotal	9.14	8.19	9.24	10.76	
Dispensable					
Alanine[c]	1.18[e]	.90[f]	1.15[e]	1.31[d]	.02
Aspartic acid	3.34	3.24	3.35	3.56	.11
Glutamic acid	5.96	5.27	5.82	6.54	.33
Glycine	2.08	2.02	2.29	2.25	.09
Serine	1.54	1.50	1.51	1.66	.05
Tyrosine	.78[de]	.68[e]	.68[e]	.91[d]	.04
Subtotal	14.88	13.61	14.80	16.23	

[a] Grams per kilogram dry matter intake.
[b] Standard error of the mean.
[c] Quadratic effect (P<.05).
[d,e,f] Values in the same row with different superscript letters differ (P<.05).

[illegible]

[illegible]

[illegible]

[illegible]

The objective of this study was to determine the effects of dietary fish (whole) on the apparent (ileal and fecal) digestibilities of crude protein and amino acids in Atlantic fish meal-based grow-finish diets in relation to the dietary fish (whole) fish meal included at four levels (0% of the experimental diets) in a 50:50 crude protein inert chromic-based oxide (Celite®) diet.

2. Materials and Methods

[illegible] at 10.1%, to possibly improve the palatability of the diets. Psyllium, as a source of fiber, was included in the diets at a level of 5.1% to facilitate defecation. Bacitracin, chromium and minerals were supplemented to meet or exceed NRC (1994) standards. Chromic oxide was used as marker to determine the digestibilities of the parameters that were measured.

Chemical and statistical analyses were carried out according to the same procedures as described in chapter 3. The gastric chemical composition and amino acid content of the experimental diets are presented in Table 4-4.

4. Results and discussion

The diet [illegible] [illegible] [illegible]

inclusion. Of the indispensable amino acids, the differences ranged from 1.3 (arginine) to 2.4 (threonine) percentage units; of the dispensable amino acids from 1.2 (tyrosine) to 0.7 (glycine) percentage units. These results are in agreement with studies reported with growing pigs and /or finishing pigs. Jongbloed et al. (1985) reported small increases in the ileal digestibilities of crude protein, methionine and threonine in diets in which the dietary level of fat was increased from 3 to 12%. Imbeah and Owen (1991) in studies with growing pigs fed corn starch-based soybean meal diets also reported a significant increase in the ileal digestibilities of most of the indispensable amino acids when the level of inclusion of canola oil was increased from 3 to 12%. Of the indispensable amino acids, the increase in apparent ileal amino acid digestibilities ranged from 1.3 to 2.3 percentage units for arginine and threonine, respectively. The response to canola oil inclusion was also evident when the pigs were fed a corn starch-based canola meal diet.

In some studies in which the effect of addition fatty acids on the apparent ileal amino acid digestibility was studied, the pigs were fed a corn starch-soybean meal diet containing 5% fat. Fan et al. (1994) found an increase in the ileal digestibilities of most amino acids when 5% tallow was added to the diet. Similarly, Li et al. (1990) reported increases in the ileal digestibilities of most amino acids when 5% soybean oil was added to the diet. Fan et al. (1994) also found that the addition of tallow increased the ileal digestibilities of most amino acids in a corn starch-soybean meal diet.

of passage of the diet in the small intestine. In turn, a slower rate of passage could result in higher amino acid digestibilities, on the assumption that the time required for the digestion of protein and/or absorption is a limiting factor. However, studies by Zebeah and Sauer (1991) found no effect of the level of dietary inclusion of fat (from 2 to 6 to 10%) on rate of passage measured at the distal ileum.

As was the case with results reported in chapters 2 and 3, the ileal digestibilities of arginine and lysine were relatively high whereas those of threonine and glycine were relatively low. These results are in agreement with studies with growing pigs fed corn starch or cereal-based soybean meal diets (Holmes et al., 1974; Sauer et al., 1983; Stein et al., 1999; Sauer et al., 2000). Possible reasons for the relatively low and high apparent ileal digestibilities of certain of the amino acids were previously discussed in chapter 3.

In contrast to the ileal analysis method, there was no effect of the dietary fat level on the ileal digestibilities of crude protein and amino acids (Table 4.5). These studies, as was previously discussed in chapter 3, show once more the improved sensitivity of the ileal compared to the fecal analysis method.

Additional reports on the effect of fat on ileal protein digestibility agree. However, in the literature, only Sauer et al. (1989) fed diets containing 2 or 10% fat and found no effect of fat on ileal digestibility of protein and amino acids.

differences (P>.05) in protein digestibility when 10% fat was included in the diet. Furthermore, no differences were found in protein digestibility in studies with growing pigs fed corn soybean meal diets that contained 0, 5, 10 or 15% fat (Greely et al., 1964). On the other hand, Asplund et al. (1960), in studies with piglets weaned at 3 weeks fed a corn-based soybean meal, meat scraps and brewers' yeast diet, observed an improvement (P<.05) in protein digestibility when 10% fat was added to the diet. Jorgensen et al. (1965), in studies with growing pigs, also observed an increase (P<.05) in the apparent protein digestibility when the dietary fat content was increased from 3 to 12%. As far as amino acid digestibilities were concerned, Deboer and Omaye (1981) in studies with growing pigs also observed no differences (P>.05) between pigs fed corn starch-based soybean meal diets that contained 5, 8 or 10% canola oil.

The disappearance of dry matter, crude protein and amino acids in the large intestine, expressed in percentage units, is presented in Table 6-8. As was expected in the previous studies (chapters 3 and 4), threonine and glycine disappeared to the largest extent. There was a decrease in the disappearance of crude protein and amino acids in the large intestine as the dietary fat level was increased from 5.0 to 10.0%. The effect is a direct result from the increase in ileal crude protein and amino acid digestibilities with increasing dietary fat level because the corresponding ileal

digestibilities remaind similar.

The disappearance of the parameters measured in the large intestine, expressed quntitatively as grams per kilogram dry matter intake, is presented in Table 4-6. As was reported previously in chapters 2 and 3, of the indispensable amino acids the disappearance was largest for leucine and threonine; of the dispensable amino acids, these were largest for aspartic acid, glutamic acid and glycine.

The average of the apparent ileal as well as fecal digestibilities of dry matter, crude protein and amino acids of the experimental diets for each experimental period are presented in Table 4-7. As was the case for the results reported in chapter 2, the apparent ileal digestibilities of the majority of the amino acids were higher ($P<.05$) in period 2 than in period 1. The differences ranged from .7 to 3.9 percentage units. The apparent fecal digestibilities of the majority of the amino acids were also higher in period 2; the differences were significant in some instances.

In summary, there was a linear increase ($P<.05$) in the apparent ileal amino acid digestibilities in soybean meal as the dietary level of cane oil was increased from 0 to 10%. The increases, however, were of a small magnitude.

TABLE 4-1. FORMULATION (%) OF DIETS WITH DIFFERENT LEVELS OF CANOLA OIL (Exp.3)

	Level of canola oil (%)			
	3.2	6.2	9.2	12.2
Ingredients:				
Soybean meal	47.8	47.9	47.9	47.9
Corn starch[a]	28.9	25.8	22.8	19.8
Canola oil	3.2	6.2	9.2	12.2
Dextrose[b]	10.5	10.5	10.5	10.5
Solkafloc[c]	4.3	4.3	4.3	4.3
TM salt	.4	.4	.4	.4
Vitamin premix[d]	1.0	1.0	1.0	1.0
Mineral premix[e]	1.0	1.0	1.0	1.0
Antibiotics[f]	.1	.1	.1	.1
Dicalcium monophosphate	.6	.6	.6	.6
Calcium carbonate	1.5	1.5	1.5	1.5
Chromic oxide[g]	.5	.5	.5	.5
DL-Methionine (98%)	.2	.2	.2	.2
Total	100.0	100.0	100.0	100.0

[a] ST. Lawrence Starch Company Ltd. Mississauga, Ontario, Canada.
[b] Dextrose, Corn Products, Englewood Cliffs, NJ, 07632.
[c] Brown company, Berlin, NH, 03570.
[d] The vitamin mixture provided the following per kg diet: 3300 IU vitamin A; 330 IU vitamin D; 24 IU vitamin E; 0.75 mg vitamin K; 5.25 mg riboflavin; 22.5 mg niacin; 15 mg pantothenic acid; 0.026 mg vitamin B_{12}; 1.5 mg thiamine; 2.25 mg vitamin B_6; 0.075 mg biotin; 0.45 mg folacin; 750 mg choline.
[e] The mineral mixture provided the following per kg diet: 150 mg Fe; 150 mg Zn; 9 mg Cu; 0.21 mg I; 0.3 mg Se.
[f] 200 g/t provides Aureomycin 100 g/t, Sulfamerazine 100 g/t and Penicillin 50 g/t.
[g] Chromic oxide powder, Fisher Scientific, Fair Lawn, NJ, 07410.

TABLE 4-2. CHEMICAL ANALYSIS AND PARTIAL AMINO ACID CONTENT (%)[a] OF DIETS WITH DIFFERENT LEVELS OF CANOLA OIL (Exp.3)

	Level of canola oil (%)			
	3.2	6.2	9.2	12.2
Dry matter	89.1	89.4	89.9	90.3
Crude protein	25.3	25.6	25.1	25.0
Neutral-detergent fiber	7.9	7.9	7.9	8.0
Acid-detergent fiber	6.4	6.4	6.5	6.5
Ether extract	4.0	7.5	11.0	13.9
Amino acids:				
Indispensable				
Arginine	1.85	1.82	1.86	1.50
Histidine	.67	.69	.67	.68
Isoleucine	1.29	1.20	1.24	1.23
Leucine	1.97	1.98	1.96	1.94
Lysine	1.73	1.74	1.76	1.77
Phenylalanine	1.30	1.24	1.27	1.24
Threonine	1.13	1.09	1.10	1.11
Valine	1.22	1.19	1.19	1.22
Dispensable				
Alanine	1.20	1.15	1.17	1.16
Aspartic acid	2.84	2.82	2.81	2.83
Glutamic acid	5.46	5.32	5.36	5.36
Glycine	1.35	1.29	1.33	1.32
Serine	1.65	1.60	1.60	1.63
Tyrosine	.75	.73	.77	.73

[a] Dry matter basis.

TABLE 4-3. APPARENT ILEAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF CANOLA OIL (Exp.3)

	Level of canola oil (%)				
	3.2	6.2	9.2	12.2	SE[a]
Dry matter	71.2	69.7	72.6	72.3	.58
Crude protein[b]	80.7[d]	81.3[d]	83.4[c]	83.3[c]	.41
Amino acids:					
Indispensable					
Arginine[b]	90.4[d]	90.8[cd]	92.1[c]	91.8[c]	.31
Histidine[b]	86.9	88.2	88.7	89.2	.47
Isoleucine[b]	85.6[d]	85.1[d]	86.8[c]	87.0[c]	.26
Leucine[b]	83.6[d]	84.1[cd]	85.2[cd]	85.5[c]	.36
Lysine[b]	85.4[d]	86.8[d]	88.6[c]	88.6[c]	.57
Phenylalanine[b]	84.7[d]	84.4[d]	86.3[c]	86.2[c]	.33
Threonine[b]	77.8[d]	79.1[cd]	80.4[cd]	81.4[c]	.63
Valine[b]	82.6[d]	82.5[d]	84.2[c]	84.9[c]	.33
Dispensable					
Alanine[b]	82.0[d]	82.1[d]	83.9[cd]	85.0[c]	.42
Aspartic acid[b]	83.7	83.5	86.1	85.9	.55
Glutamic acid[b]	85.9[d]	87.7[cd]	90.5[c]	90.2[c]	.65
Glycine[b]	77.9[d]	78.3[d]	82.4[c]	83.5[c]	.47
Serine	87.5[d]	87.0[d]	88.6[c]	88.5[c]	.19
Tyrosine[b]	85.1[c]	86.1[bc]	87.1[cd]	87.6[c]	.28

[a] Standard error of the mean.
[b] Linear effect (P<.05).
[c,d] Values in the same row with different superscript letters differ (P<.05).

TABLE 4-4. APPARENT FECAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF CANOLA OIL (Exp.3)

	Level of canola oil (%)				
	3.2	6.2	9.2	12.2	SE[a]
Dry matter	89.1	88.8	89.2	87.6	.58
Crude protein	89.5	90.1	90.0	90.1	.67
Amino acids:					
Indispensable					
Arginine	95.4	95.9	96.0	96.0	.42
Histidine	94.4	95.4	94.9	95.2	.38
Isoleucine	89.4	90.4	90.3	90.3	.66
Leucine	89.4	90.7	90.6	90.7	.63
Lysine	91.1	92.1	92.1	92.5	.65
Phenylalanine	90.6	91.3	91.6	91.5	.66
Threonine	88.7	89.1	89.7	89.5	.72
Valine	88.0	89.1	89.7	89.5	.72
Dispensable					
Alanine	86.5	87.3	87.9	88.0	.82
Aspartic acid	92.1	92.8	93.1	93.0	.57
Glutamic acid	95.1	95.6	95.7	95.7	.37
Glycine	90.6	91.1	91.4	91.6	.58
Serine	94.0	94.5	94.5	94.6	.42
Tyrosine	90.0	90.7	91.2	91.3	.66

[a] Standard error of the mean.

TABLE 4-5. DISAPPEARANCE[a] OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN THE LARGE INTESTINE OF PIGS FED DIETS WITH DIFFERENT LEVELS OF CANOLA OIL (Exp.3)

	Level of canola oil (%)			
	3.2	6.2	9.2	12.2
Dry matter	17.9	19.1	16.6	15.2
Crude protein	8.7	8.8	6.6	6.8
Amino acids:				
Indispensable				
Arginine	5.0	5.2	3.9	4.3
Histidine	7.5	7.2	6.3	6.1
Isoleucine	3.8	5.3	3.5	3.3
Leucine	5.9	6.6	5.5	5.3
Lysine	5.7	5.3	3.5	3.9
Phenylalanine	6.0	7.0	5.3	5.4
Threonine	10.9	10.4	9.5	8.5
Valine	5.4	6.6	5.6	4.7
Dispensable				
Alanine	4.5	5.2	4.1	3.1
Aspartic acid	8.4	9.4	7.0	7.1
Glutamic acid	9.2	7.9	5.2	5.5
Glycine	12.7	12.8	9.1	8.1
Serine	6.7	7.6	6.0	6.1
Tyrosine	4.9	4.6	4.1	3.7

[a] Percentage units.

TABLE 4-6. DISAPPEARANCES[a] OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN THE LARGE INTESTINE OF PIGS FED DIETS WITH DIFFERENT LEVELS OF CANOLA OIL (Exp.3)

	Level of canola oil (%)				
	3.2	6.2	9.2	12.2	SE[b]
Dry matter	178.9	191.3	165.7	152.3	10.91
Crude protein	22.9	22.9	16.2	17.3	2.71
Amino acids:					
Indispensable					
Arginine	.92	.94	.74	.79	.11
Histidine	.51	.50	.41	.46	.05
Isoleucine	.50	.58	.41	.40	.11
Leucine	1.16	1.31	1.03	1.02	.17
Lysine	1.00	.93	.59	.69	.19
Phenylalanine	.77	.87	.67	.67	.11
Threonine[c]	1.23	1.14	1.03	1.04	.12
Valine	.66	.79	.58	.57	.12
Subtotal	6.75	7.06	5.46	5.64	
Dispensable					
Alanine	.54	.66	.45	.46	.14
Aspartic acid	2.39	2.64	1.94	2.01	.29
Glutamic acid	4.49	3.79	2.76	2.95	.75
Glycine	1.60	1.51	1.18	1.15	.19
Serine	1.07	1.21	.94	.99	.09
Tyrosine	.37	.39	.31	.33	.07
Subtotal	10.46	10.20	7.58	7.89	

[a] Grams per kilogram dry matter intake.
[b] Standard error of the mean.
[c] Linear effect ($P<.05$).

TABLE 4-7. EFFECT OF EXPERIMENTAL PERIOD ON ILEAL AND FECAL DIGESTIBILITIES (%) OF DRY MATTER, CRUDE PROTEIN AND AMINO ACIDS IN DIETS WITH DIFFERENT LEVELS OF CANOLA OIL (Exp.3)

	Ileal			Fecal		
Period	1	2	SE[a]	1	2	SE[a]
Dry matter	70.7[c]	72.3[b]	.41	87.8[c]	89.4[b]	.41
Crude protein	81.6[c]	82.8[b]	.29	88.8[c]	90.9[b]	.48
Amino Acids:						
Indispensable						
Arginine	90.5[c]	91.9[b]	.22	95.3[c]	96.2[b]	.25
Histidine	87.7	88.6	.34	94.5[c]	95.4[b]	.27
Isoleucine	85.6[c]	86.6[b]	.20	89.2	90.6	.47
Leucine	83.9[c]	85.3[b]	.25	89.5[c]	91.2[b]	.45
Lysine	86.6	88.0	.42	91.0[c]	92.8[b]	.46
Phenylalanine	84.9[c]	85.9[b]	.23	90.8	91.7	.46
Threonine	77.7[c]	81.6[b]	.44	88.5[c]	90.4[b]	.48
Valine	82.9[c]	84.2[b]	.24	88.2[c]	89.9[b]	.51
Dispensable						
Alanine	82.9	83.6	.30	86.6	88.2	.58
Aspartic acid	84.3	85.2	.39	92.1	93.4	.40
Glutamic acid	87.7	89.3	.39	95.1	95.9	.26
Glycine	79.7[c]	81.4[b]	.33	90.5	91.8	.41
Serine	87.7[c]	88.2[b]	.13	93.9	94.8	.30
Tyrosine	85.9[c]	87.0[b]	.19	90.1	91.4	.47

[a] Standard error of the mean.
[b,c] Values in the same row with different superscript letters differ ($P<.05$).

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5. GENERAL DISCUSSION AND CONCLUSION

Many studies have been carried out in the area of protein and amino acid digestibility with growing and finishing pigs. At present, there is considerable interest in amino acid digestibility studies with early-weaned starter pigs. The digestive system of the pig, weaned between three and five weeks of age, is still very immature as exemplified by the low rate of secretion of gastric HCL and some of the pancreatic enzymes (Corring et al., 1978). The secretion of digestive enzymes will reach its full potential during the first 10 weeks of life (Shields et al., 1980). Early-weaned pigs, therefore, face a severe nutritional stress resulting from the abrupt shift from milk to plant protein-based diets (usually protein from soybean meal).

In recent years, various techniques have become available to measure amino acid digestibility in small pigs using the ileal analysis method (Walker et al., 1986; van Leeuwen et al., 1988; Freire et al., 1988). As was reviewed many times during the last decade the ileal rather than fecal analysis method, for reasons of the modifying action of the microflora in the large intestine, should be used to determine amino acid digestibility (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). An adaptation of the technique by Walker et al. (1986), involving the placement of a simple T-cannula, was used in these studies. This technique was further modified by

the preparation of cannulas made from plastisol. These type of cannulas can be removed at the completion of the studies. Following the closure of the fistula, the pigs can be returned to the herd.

A series of studies were carried out to determine the influence of various dietary components (crude protein, fiber and fat) on amino acid digestibility. Corn starch-based soybean meal diets were used as "model" diets. The effect of dietary crude protein content was examined in the first experiment. Diets containing 16.4, 19.5, 22.5 and 25.5% crude protein were formulated. There was a linear decrease ($P<.05$) in the ileal digestibilities of most of the amino acids, however of a small magnitude, as the dietary protein content was increased. The small but significant decrease, as was postulated, may result from the possibility that the total supply of amino acids and peptides exceed the capability of absorption (Alpers, 1987). On the other hand, as the protein level increased, the dietary NDF and ADF contents increased from 6.9 to 8.4% and from 5.8 to 6.7%, respectively. The increased fiber content may also have affected the apparent digestibility of protein and amino acids. It is important to note that this linear decrease was not observed with the fecal analysis method indicating a modifying effect by the microflora in the large intestine. In addition, it is important to point out that there was a relatively large disappearance of amino acids, originating from endogenous

secretions, in the large intestine.

The effect of dietary fiber content on amino acid digestibility was investigated in the second experiment. Diets containing 22.5% crude protein with 4.3, 7.3, 10.3 and 13.3% Solkafloc, which was selected as fiber source, were formulated. For most of the amino acids, there were no differences ($P>.05$) in ileal digestibilities between diets containing 4.3, 7.3 and 10.3% Solkafloc. There was a decrease in the digestibilities of most of the amino acids ($P<.05$) when 13.3% Solkafloc was included in the diet. The decrease in amino acid digestibility, observed at the higher level of Solkafloc inclusion, may result from several factors including the adsorption of amino acids and peptides by the fiber, withholding these from absorption (Mitaru et al., 1984). Other factors include an increase in the sloughing of intestinal mucosal cells and enhanced mucus production (Schneeman et al., 1982). Furthermore, certain sources of fiber have gel-forming properties creating an environment in the digestive tract which impairs the hydrolysis of protein (Murray et al., 1977). The aforementioned factors that may result in a measurable increase in amino acid losses during digestion may only take on quantitative importance following the inclusion of particular level of fiber in the diet (threshold level); the exact level will be dependent on the source of fiber, the age of the animal and other factors.

The effect of dietary fat content on amino acid

digestibility was investigated in the third experiment. Diets containing 22.5% crude protein with 3.2, 6.2, 9.2 and 12.2% canola oil were formulated. There was a linear increase ($P<.05$) , however of a small magnitude, as the inclusion level of canola oil was increased. It is rather difficult to explain the effect of fat on ileal amino acid digestibility. The improvement does not result from a decrease in the recovery of endogenous amino acids at the distal ileum (de Lange et al., 1989) or a decrease in the rate of passage of the diet (Imbeah and Sauer, 1991). Based on results obtained with the fecal analysis method, the improvement in amino acid digestibility resulting from additional fat could not be detected.

In summary, these studies show that the content of dietary protein, fiber and fat may affect the ileal amino acid digestibilities in diets for early-weaned pigs.

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